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RESEARCH ARTICLE

Non-target host risk assessment of the idiobiont parasitoid *Bracon celer* (Hymenoptera: Braconidae) for biological control of olive fruit fly in California

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Non-target risk posed by an African parasitoid, *Bracon celer* Szépligeti (Hymenoptera: Braconidae), was assessed for a classical biological control program against olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae: Dacinae), in California, USA. Behavioral and reproductive responses to non-target tephritid species were tested with beneficial (*Chaetorellia succinea* [Costa] and *Parafreutreta regalis* Munro) (Tephritidae: Tephritinae) and native (*Rhagoletis fausta* [Osten Sacken]) (Tephritidae: Trypetinae) fruit fly species in successive no-choice and choice experiments under close confinement in quarantine. Non-target host-plant substrates exposed to *B. celer* were yellow-starthistle flower heads containing *C. succinea*, Cape ivy stem galls containing *P. regalis*, and bitter-cherry fruit containing *R. fausta*. The parasitoid probed all three infested non-target plant substrates, but significantly less than olives infested with *B. oleae*. It produced offspring from *P. regalis* in Cape ivy stem galls, but appeared unable to penetrate yellow-starthistle flower heads with its ovipositor. *Bracon celer* killed some *B. oleae* and *R. fausta* larvae without parasitism. Reproduction on *P. regalis* indicates that *B. celer* has a broad physiological host range, which, combined with the parasitoid's acceptance of all three host-plant substrates, indicates a strong potential to negatively impact non-target species. Although physical and temporal barriers to host attack may reduce risk to most non-target tephritids by *B. celer* in California, the parasitoid should not be released due to its risk of harming the beneficial *P. regalis*. Release of *P. regalis* is still under consideration, however, and final risk assessment should depend on whether the fly proves useful for weed control.

Keywords: olives; biological control; non-target risk; *Bactrocera oleae*; *Bracon celer*; Tephritidae

Introduction

Bracon celer Szépligeti (Hymenoptera: Braconidae) is one of the more abundant parasitoids attacking olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae),

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in wild and commercial olives in South Africa (Neuenschwander 1982; Walton, Daane, and Stotter 2005) and Kenya (Silvestri 1914; but see Copeland, White, Okomu, Machera, and Wharton 2004). In one study, it was reported to achieve 87% parasitism of *B. oleae* in South African orchards (Annecke and Moran 1982). Recently, *B. celer* was imported from Africa to California, USA, for evaluation as a classical biological control agent for release against *B. oleae* (Sime et al. 2006a), along with other African (Sime, Daane, Messing, and Johnson 2006b; Daane et al. 2008) and Asian (Sime et al. 2007) parasitoids of *B. oleae*. *Bactrocera oleae* is native to Africa and Asia and is also found in Europe, where it is a serious pest of olives. The fly was discovered in California in 1998 and spread rapidly to olive growing regions throughout the state (Yokoyama, Miller, Stewart-Leslie, Rice, and Phillips 2006). *Bracon celer*, a member of the subfamily Braconinae, develops as a solitary, ectoparasitic idiobiont on third (last) instar *B. oleae* inside fruit (Neuenschwander 1982; Sime et al. 2006a). As such, it differs from other braconid species naturally attacking *B. oleae* in its native range. These are comprised of solitary, endoparasitic, larval-pupal koinobionts (subfamily Opiinae) that deposit their eggs into more than one instar (Sime et al. 2006b,c, 2007; Daane et al. 2008). Efforts to use *B. celer* against *B. oleae* in Europe were unsuccessful. The parasitoid could not be reared (Silvestri 1914; Neuenschwander 1982), and a release of field-collected adults shipped from South Africa to Greece failed to establish (Wharton 1989). After importation to the Californian quarantine facility of the College of Natural Resources, University of California, Berkeley, *B. celer* was successfully reared in colony for 6 months on *B. oleae* in olives until maturing olive quality became too poor to sustain the parasitoids (Sime et al. 2006a).

Bracon celer appeared to be oligophagous, having also been reared from Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) in Kenya (Wharton et al. 2000), while an unconfirmed record exists from *C. nigra* Graham (= *Trirhithrum coffeae* Bezzi) (Narayanan and Chawla 1962). These records are from coffee berries in the field. The parasitoid was reared in southern Africa from olive samples containing both *B. oleae* and *B. biguttula* (Bezzi), posing the possibility that it attacks *B. biguttula* (Mkize, Hoelmer, and Villet 2008). We suspected that *B. celer*'s host range may be even wider than reported. Surveys of tephritid parasitoids in Africa have historically concentrated on frugivorous tephritids likely to yield natural enemies of crop pests, but little information has been gathered on parasitoids of non-frugivorous African tephritids that could serve as hosts. More importantly, *B. celer*'s ectoparasitic idiobiont lifestyle is typical of parasitoids that exhibit low host specificity. In contrast to koinobionts, idiobionts are under less selection pressure to adapt to a host's physiology, and may successfully utilize several host families and orders, often acting as facultative hyperparasitoids (Shaw 1994; Althoff 2003). There was a strong possibility therefore, that *B. celer* is polyphagous and capable of attacking non-target species in California.

Attack of non-target hosts is of concern due to the potential harm that exotic natural enemies may impose on native or beneficial exotic species (Hoddle 2004; Messing and Wright 2006; van Lenteren, Bale, Bigler, Hokkanen, and Loomans 2006a). However, risk to non-target species is complex and difficult to estimate, especially in quarantine facilities where imported natural enemies are sequestered as they are being evaluated for release. Guidelines for appropriate standardized tests and interpretations of results are being developed and tested (van Lenteren, Cock,

Hoffmeister, and Sands 2006b). However, limited space and resources, in addition to often untenable numbers of potential hosts, can hamper efforts to accurately assess non-target risk, especially when the target pest is an arthropod (Messing 2001). Quarantine screening processes for parasitoids have traditionally tested little more than a non-target species physiological suitability, as opposed to its ecological suitability, which is more meaningful in the field (Messing and Wright 2006). A test of ecological suitability necessarily includes study of orientation toward host habitat, a critical step in the hierarchy of behaviors leading to successful parasitism, but this requires use of replicated large enclosures whose use may be discouraged under limited quarantine conditions. Tests of physiological suitability have contributed to good safety records of recent natural-enemy importations, although reliance solely on such conservative tests can lead to rejection of some potentially useful biological control agents (Messing and Wright 2006). A capacity to attack individuals of a non-target species should also not be simplistically interpreted as leading to significant population-level changes in the non-target species (Messing and Wright 2006; Frank and McCoy 2007).

Natural enemy introductions to combat tephritid pests in California could possibly impact a rich tephritid fauna. California is home to over 120 native tephritids, several of which are rare and/or endemic (Foote, Blanc, and Norrbom 1993). Several exotic tephritid species were also imported to California to combat invasive weeds and are now established or under consideration as biological control agents. Among these is a guild of fruit flies, including the Australian species *Chaetorellia succinea* (Costa), that aids in combating a noxious Mediterranean weed, the yellow starthistle (*Centaurea solstitialis* L.) (Asteraceae) (Pitcairn, Schoenig, Yacoub, and Gendron 2006). Another imported beneficial tephritid is the South African *Parafreutreta regalis* Munro, which is under quarantine evaluation for release in California against Cape ivy (*Delairea odorata* Lemaire) (Asteraceae), an invasive South African weed (Balciunas and Smith 2006). Yellow starthistle commonly grows in disturbed areas bordering roadside olive trees and commercial olive groves throughout central and southern California, a situation that will promote encounters between imported parasitoids of *B. oleae* and beneficial tephritids used for starthistle control. Likewise, the coastal habitat of Cape ivy overlaps with olives in urban settings. We therefore studied the capacity of *B. celer* to attack and reproduce on these tephritids and evaluated whether its release would threaten these beneficial species. Among the native tephritids that may be threatened by introduced *B. oleae* parasitoids, none are closely related to *B. oleae*, which is a member of the tribe Dacini in the subfamily Dacinae. The native species of California are placed in the subfamilies Trypetidae and Tephritinae, which are the only subfamilies naturally occurring in North America. Larvae of Trypetinae feed in various plant structures, including fruit, while larvae of the Tephritinae are almost entirely restricted to feeding within galls and flower heads of members of the sunflower family, Asteraceae (Foote et al. 1993).

Faced with a large number of potential non-target host species, our approach was to assess *B. celer*'s capacity to attack and reproduce on non-target tephritid species inhabiting common types of host-plant substrates and representing a sample of the broad phylogenetic diversity found in California (several tribes within both North American subfamilies of Tephritidae). We confined the parasitoid with infested plant material in small cages to provide a conservative assessment of host suitability, and

compared its behavior and reproduction in sequential no-choice and choice studies with the target host. The non-target hosts we selected were the beneficial species *C. succinea* and *P. regalis* and the native black cherry fruit fly, *Rhagoletis fausta* (Osten Sacken), a trypetine frugivore that infests fruit of bitter cherry, *Prunus emarginata* (Douglas ex Hooker) Eaton (Rosaceae) in the mountains of California.

Materials and methods

Insect and plant colonies

Separate colonies of *B. oleae* and *B. celer* were established at the University of California, College of Natural Resource's Quarantine in Berkeley. *Bactrocera oleae* were reared on olive fruit using methods described by Sime et al. (2006). Adult flies were held in ventilated cages (50 × 50 × 50 cm) provisioned *ad libitum* with water and a 2:1 mixture of honey and dry yeast extract (Fisher Biotech, Fairlawn, NJ, USA). Susceptible olives were exposed to the fly colony until each fruit had 5–10 oviposition marks, typically <1 day, and then removed to a separate rearing cage. Infested olives were held until mature fly larvae exited the fruit to pupate, upon which the puparia were collected and transferred to Petri dishes. Small to medium-size olives (cv. Mission or Manzanilla), collected from orchards in Fresno County, were used for all experiments, while olives of various cultivars, including Ascolana and Sevillano, were also used for maintenance of the fly and parasitoid colonies.

The *B. celer* colony originated from wild olives (*Olea europaea* L. ssp. *cuspidata* [Wall. ex G. Don]) collected in West Cape Province, South Africa, and Otjozondjupa Region, Namibia, in April and May 2004. The olives were shipped by air to a quarantine facility at the European Biological Control Laboratory in Montferrier, France. Eight female and seven male *B. celer* from that field collection were sent to the Berkeley Quarantine in June 2004, and studies commenced with the first generation of offspring. Adults were held in ventilated cages (45 × 45 × 45 cm) freely provisioned with fly-infested olives, water, and a honey–water solution (50:50%, v/v). Host larvae included both second and third instar *B. oleae* produced from ovipositions 8–10 days earlier (Sime et al. 2006a). Olives were exposed to wasps for 1–3 days and then transferred to another cage for parasitoid emergence.

The *P. regalis* colony was maintained at the Berkeley Quarantine in summer and fall 2004 for non-target host assessment. The colony was established from stock (originally from South Africa) maintained at the USDA–ARS quarantine facility in Albany, California. Rearing methods used are described by Balciunas and Smith (2006). In brief, potted Cape ivy plants were placed in 32 × 45 × 96 cm sleeve cages with adult *P. regalis* for 4–7 days and then removed to racks under filtered daylight for gall maturation. Mature galls were cut from the plants with a few centimeters of stem for use in tests or placed in moist blocks of Oasis® floral foam (Smithers-Oasis, Cuyahoga Falls, OH, USA) in cages for colony adult emergence.

A second non-target tephritid species, *C. succinea*, was produced using a rearing system based on that described in Balciunas and Villegas (2001). Adult *C. succinea* were reared from yellow starthistle flower heads collected in Contra Costa and Yolo counties from July through October 2004. Emerged adult flies were caged and supplied with water and a mixture of dry yeast extract and honey. To produce flower heads infested with groups of same-aged larvae, potted plants were pruned of all but

the suitable flower buds, up to a week before expected anthesis, and placed in a cage with adult flies (6–14 days old) for 2 days. The plants were then moved to a greenhouse ($23 \pm 3^\circ\text{C}$) to rear the larvae, which were used in non-target studies when they matured to third instar.

The third non-target species, *R. fausta*, is a univoltine species found in ripening bitter cherry fruit in late summer and fall. Branches bearing fruit with evidence of *R. fausta* infestation (oviposition scars) were collected on the western slope of the Sierra Nevada mountains (Fresno County) in August and September 2004 and brought in secured coolers to the Berkeley Quarantine for immediate use in experiments. We did not establish a colony of *R. fausta* because this species is univoltine and adults emerge about 10 months after diapausing in the pupal stage. The field-collected larvae we used were, therefore, potentially parasitized by native Opiine braconids in the genera *Diachasma* and *Utetes* (Wharton 2008), which are internal, larval-pupal koinobionts. Testing the effect of prior host parasitism by native koinobionts on *B. celer* was outside the scope of our work, but parasitoids are usually incapable of recognizing prior parasitism by other species (Boivin and Brodeur 2006). Furthermore, prior parasitism by an internal koinobiont is thought to have little effect on host acceptance or reproduction by an idiobiont (Godfray 1994; Mayhew and Blackburn 1999), and empirical tests of this hypothesis so far support it (Mitsunaga and Yano 2004). Because all or most *R. fausta* in each replicate were later found to be free of parasitism by native parasitoids, we were thus able to meet our goal of testing whether *B. celer* could develop on this tephritid.

Voucher specimens of *B. celer* were deposited at U.C. Berkeley Quarantine, referenced with the Shipment and Receiving Code 2004-10B. Samples of tephritids reared during the study are deposited in the same collection and are labeled Daane Olive Fly Project.

Assessment of host range

Non-target species were first offered to *B. celer* without choice, after which the target host was provided alongside the non-target species to the same *B. celer* individuals, serving both as a choice test with *B. oleae* and as a positive control to ensure the readiness and ability of tested parasitoids to oviposit (Van Driesche and Murray 2005). Behavioral observations were made to record the wasp's responsiveness and readiness to accept the fly larva within the plant substrate, while the physiological suitability of the fly species was determined by rearing. Non-target fly species belonging to three tribes within two subfamilies were offered to test the breadth of the parasitoid's physiological host range, while diverse infested plant substrates were offered to provide information on the parasitoid's propensity to recognize, search, and accept a variety of non-target larval substrates. Because no native Dacinae occur in North America, tephritids closely related to *B. oleae* were not tested, but host records from *C. capitata* in the field (Wharton et al. 2000) sufficed to show that *B. celer* is capable of locating and developing in a frugivorous member of the dacine tribe Ceratitini in addition to the tribe Dacini.

Experimental non-target hosts selected from the Trypetinae and Tephritinae utilize the three most common tephritid host-plant substrates in North America: fruits, flower heads, and stem galls. Species selection was based on a set of criteria aimed to maximize both practicality and potential for host acceptance, including

ease of locating and/or rearing host plants and hosts, comparable sizes of non-target and target hosts, and resemblance of host-plant substrates (e.g., galls) to olives in shape and size. Adequate host size aimed to avoid unintended parasitoid offspring mortality due to insufficient host resources, while the size of the host-plant substrate (smaller than or equal to olives) aimed to minimize negative results caused by host inaccessibility, i.e., hosts that are buried beyond the reach of a parasitoid's ovipositor. *Chaetorellia succinea*, which feeds on developing seeds inside yellow starthistle flower heads, and *P. regalis*, which forms spherical galls in Cape ivy stems, both conform to this set of criteria and were chosen also to directly address the risk posed to beneficial species by the candidate parasitoid. *Chaetorellia* and *Parafeutreta* belong to the Tephritinae tribes Terellini and Tephritini, respectively, which have native representatives in California. The third species that conforms to selection criteria, the black cherry fruit fly, *R. fausta*, was chosen as a native frugivore representative of the Trypetinae (tribe Carpomyini).

Non-target tests were conducted in Quarantine using small wood cages ($25 \times 25 \times 25$ cm) with glass fronts and screen sides. The two phases of each test consisted of a no-choice phase in which parasitoids were offered only the non-target material for 2 days, and a positive control phase in which olives infested with *B. oleae* were added to the cages with the original non-target material for an additional 2 days (Figure 1). Additional cages were set up as negative controls at each trial date, in which non-target hosts and *B. oleae* were set up concurrently with the no-choice tests and positive controls, but parasitoids were excluded. Negative controls provided information on baseline host emergence and mortality rates under laboratory conditions. These negative controls were randomly selected plant substrates from the

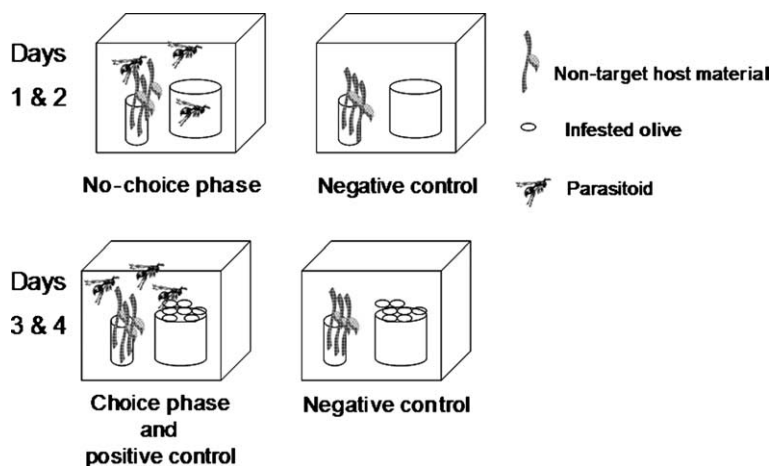


Figure 1. Diagram of experimental procedure, showing one test replicate and a negative control. The test is started on Day 1 with a no-choice phase, where infested non-target plant material is placed in a cage with female parasitoids, and a subsample of the non-target material is placed into a cage without parasitoids (negative control). On Day 3, infested olives are added to the cage with parasitoids and the negative control. Each phase lasts 48 h. The number of wasps performing behaviors classed as 'incidental contact', 'investigation', and 'probing' on the infested plant structure is recorded during three 10-min observation periods.

same batches of material exposed to *B. celer* on any particular date. All plant materials were incubated for at least 6 weeks after the trials to allow for adult parasitoid or fly emergence.

Non-target fly larvae were presented *in situ* (in flower heads, galls, or fruit) in bouquets 10–20 cm long, with the bases in water and leaves removed to allow a clear view of wasp behaviors (for observations described below). Buds of yellow starthistle had been exposed to adult *C. succinea* 10–12 days earlier. Dissection of subsamples revealed that 76% of the buds were infested and that over 60% of larvae had reached third instar. Cape ivy galls were used 20–27 days after exposure to *P. regalis*, when dissections revealed that 80% of larvae were in third instar. Bitter cherry infestation was estimated at only 14% of fruit, and of these, 80% of the larvae were in the third instar. The remaining hosts were in the second instar when the studies began, allowing new third instars to be recruited during the second (choice) phase of the trials.

In the first (no-choice) phase of the test, a bouquet of non-target plant material was placed in each cage. The amount of parasitoid and host material in each replicate depended on availability of material, but an effort was made to present similar masses of non-target and olive material. Because the sustainability of the parasitoid colony was questionable and numbers were initially very low, we began using colony females (i.e., experienced on *B. oleae* in olives) for host-specificity testing from the first reared generation, and continued using colony females as our standard. Between 10 and 15 female *B. celer* were added to each cage provided with 8–10 galls (*P. regalis*), 10–20 flower heads (*C. succinea*), or 20 fruit (*R. fausta*). Each replicate began between 08:00 and 09:30 h. The second (choice or positive control) phase began at 09:00 h on the third day, when 8–10 olives (Manzanillo, Mission, or Sevillano cultivars) infested with 5–10 third instar *B. oleae* were placed in the cage at a height level with the non-target material. This phase lasted an additional 2 days.

On the first day of both the no-choice and choice phases, the parasitoids were observed for 10 min at 10:00, 13:00, and 16:00 h, and the numbers that were in contact with the host-plant substrate (i.e., flower head, gall, or fruit) were recorded. Records were made in three behavioral categories: investigating, probing, or incidental contact. Investigating was defined as a walk with slightly lowered antennae, interrupted by frequent stops with the antennae raised, presumably while locating hosts through vibrotaxis, a strategy common in parasitoids searching for hidden hosts (Godfray 1994; Canale and Loni 2006). Probing was always preceded by this behavior. Probing was defined as the insertion or attempted insertion of the ovipositor into the host-plant substrate, and both successful and unsuccessful penetration were included under probing in analyses. Investigation that led to probing by an individual during the observation period was recorded under both categories. Other behaviors on the flower head, fruit, or gall that did not appear to be associated with searching or probing, such as standing motionless, grooming, or brief contact, were recorded as incidental contact. A secondary purpose of the behavioral observations was to ascertain if absence of parasitoid offspring resulted from no contact with the host substrate, or was due to host rejection or unsuitability.

At the end of the choice phase, plant materials and associated hosts were separated by species and incubated for a minimum of 6 weeks. Bouquets of Cape ivy galls, still maintained on stems in water, were confined in vials with mesh windows, while flowers and fruit were removed from stems and held in plastic vials (flowers) or

paper cups (fruit) with mesh lids. Pupation occurred inside flowers and galls, but outside cherries. Pupation occurred both inside and outside olives. The numbers of adult flies and parasitoids that emerged were recorded. Galls, cherries, and flower heads were later dissected and the numbers of dead larvae, pupae, and adults inside were recorded. Olive fruit were not dissected unless adult *B. celer* failed to emerge from a positive control. The negative controls were treated in a similar manner. Differentiation between larvae and pupae of *B. celer* and those of native parasitoids from cherry fly puparia was done by color, as the immature stages of native wasps were white, markedly different from the gray to yellow *B. celer* larvae and pupae (HN, personal observation).

Statistical analyses

Numbers of wasps engaged in three behavior classes (incidental contact, investigation, and probe) are presented as means (\pm SEM) for each of the three test treatments: non-target without choice, non-target with choice, and target with choice. Treatment means were compared for each host species using one-way ANOVA followed by Tukey's HSD test to separate the means. Proportional parasitism data are presented as treatment means (\pm SEM) and were normalized by arcsine-square-root-transformation before paired *t*-test analysis to compare non-target and positive-control (*B. oleae*) groups. Mean numbers per fruit of *B. oleae* that survived after exposure to *B. celer* (positive control) and that were not exposed (negative controls) were normalized by log-transformation and compared by paired *t*-test. Counts of dead third instars in groups of *P. regalis* and *R. fausta* exposed or not exposed to *B. celer* were compared with Fisher's exact test. In all cases $\alpha = 0.05$. All data analyses were performed using Prism 5.01 (GraphPad Software, Inc., San Diego, CA, USA).

Results and discussion

Under the close confinement imposed by the experimental design, *B. celer* responded to all non-target and target species by investigating and probing, or attempting to probe, the infested plant substrates during both no-choice and choice phases of the tests (Figure 2). Attempts to probe into starthistle flower heads failed, probably because the involucre bracts at the base of the flower head prevented penetration by the ovipositor. Parasitoid offspring were produced from the target host and from *P. regalis* (Table 1). The numbers of wasps observed investigating and probing non-target host substrates were significantly lower than on the target substrate during comparisons of both the 2-day no-choice phase and the 2-day choice phase of the tests (Figure 2). Incidental contact by wasps (i.e., contact with the gall, fruit, or flower head but without behaviors associated with searching or probing) occurred least on olives, presumably because any contact with infested olives immediately elicited investigation behavior, but was significantly less only on olives compared with starthistle flower heads when a choice was presented of both substrates. The mean proportion of parasitized *P. regalis* (0.11 ± 0.04) was significantly lower than parasitized *B. oleae* (0.54 ± 0.07) (paired *t*-test, $t = 5.238$; $df = 6$; $P = 0.002$). Only one of 14 *B. celer* offspring reared from *P. regalis* was female, whereas the sex ratio of

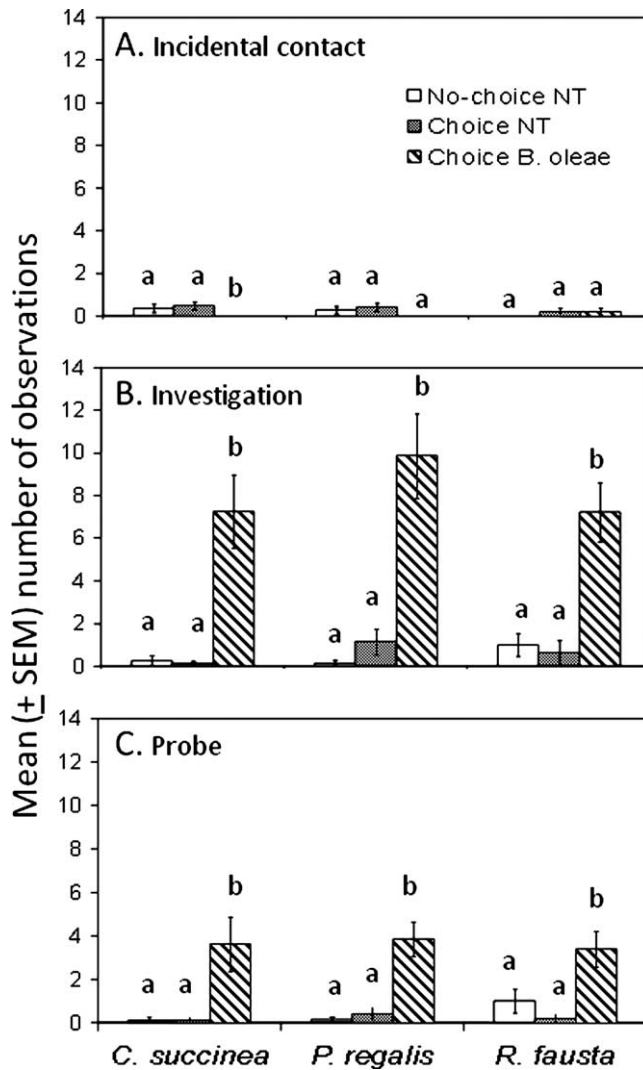


Figure 2. Comparison of behavioral responses of female *B. celer* to non-target (NT) hosts and *B. oleae* recorded during three 10 m observation periods in a 48 h no-choice phase (only NT) and three 10 m observation periods during a subsequent 48 h choice phase (NT and *B. oleae*). The responses were classed as: (A) incidental contact with infested host-plant substrates without any apparent response to hosts; (B) investigation behavior indicating awareness of host presence; and (C) probing the substrate with the ovipositor. The non-target hosts were *Chaetorellia succinea* in yellow starthistle flower heads, *Parafreutreta regalis* in Cape ivy stem galls, and *Rhagoletis fausta* in bitter cherry fruit. *Bactrocera oleae* were offered in olives. Different letters above each group of bars (species) indicate significant differences (ANOVA, incidental contact: *C. succinea* $F=23.48$; $df=2,21$; $P<0.001$; investigation: *C. succinea* $F=131.6$; $df=2,21$; $P<0.001$; *P. regalis* $F=19.2$; $df=2,18$; $P<0.001$; *R. fausta* $F=15.8$; $df=2,12$; $P=0.0004$; Probing: *C. succinea* $F=59.9$; $df=2,21$; $P<0.001$; *P. regalis* $F=17.13$; $df=2,18$; $P<0.001$; *R. fausta* $F=8.32$; $df=2,12$; $P=0.005$; all followed by Tukey's HSD, $P<0.05$).

Table 1. Number of fly and parasitoid offspring reared from flies exposed to *B. celer* and from negative controls.

Non-target species	Offspring reared from flies exposed to parasitoids (no-choice and choice phases)					Offspring reared from negative control			
	Non-target flies			<i>B. oleae</i>		Non-target flies		<i>B. oleae</i>	
	<i>n</i> ^a	Flies ^b	Parasitoids ^c (% parasitism)	Flies	Parasitoids (% parasitism)	<i>n</i>	Flies	<i>n</i>	Flies
<i>C. succinea</i>	8	143	0 (0.0)	147	109 (42.6)	7	94	7	388
<i>P. regalis</i>	7	80	14 (14.9)	185	187 (50.3)	4	41	4	466
<i>R. fausta</i>	6	14	0 (0.0)	35	192 (84.6)	4	35	4	265

^a*n*, number of replicates.
^bFlies, number of fruit fly puparia and adults.
^cParasitoids, number of emerged adults and adult and immature *B. celer* cadavers.

B. celer reared from *B. oleae* was 1.3:1 males to females, similar to the average colony sex ratio (1.2:1) reported by Sime et al. (2006a). Despite probing into cherries, *B. celer* produced no offspring from this substrate, leading us to conclude that it cannot reproduce on *R. fausta*. Upon dissection of *R. fausta* puparia, one of the bitter-cherry replicates was found to have prior parasitism by native braconid parasitoids. In this case, 38% of *R. fausta* puparia contained solitary diapausing pupae of a native braconid. No *B. celer* eggs or larvae were observed on these pupae or on fly larvae, but eggs may have escaped detection due to deterioration over the 6 wk incubation period. All *R. fausta* replicates contained unparasitized larvae that could have served as primary hosts for *B. celer*. *Bracon celer* offspring were produced from *B. oleae* in all but one positive-control group (in a *C. succinea* replicate), which was excluded from the analysis.

Fly survival in negative control groups appeared to be higher than in groups exposed to *B. celer*, suggesting that the parasitoid killed some fly larvae without producing offspring. This phenomenon is documented in several parasitoid species under high wasp-to-host ratios (van Alphen and Vet 1986) and should be minimized in host specificity tests to avoid underestimating host suitability (van Lenteren et al. 2006b). When survival of exposed and unexposed third instars of *B. oleae* were compared, the number of *B. oleae* surviving after exposure to *B. celer* (5.01 ± 0.51 per fruit), was significantly lower than the number surviving in the negative controls where wasps were absent (7.54 ± 1.01 per fruit) (paired *t*-test, $t = 3.983$, $df = 14$, $P = 0.001$). For the purpose of this comparison, surviving third instar hosts are defined as those that pupated or produced an adult fly or wasp. Evidence for direct host killing without wasp reproduction was also found in *R. fausta*. A significantly greater proportion of third instar *R. fausta* (7 of 21 individuals, or 33.3%) died after exposure to *B. celer*, while none of 35 individuals died in the negative controls (Fisher's exact test, $P < 0.001$). A similar comparison of third instar *P. regalis* mortality in exposed (16 of 110 individuals, or 14.5%) and negative control groups (4 of 45 individuals, or 8.8%) was not statistically significant. It is noteworthy that some third instar *P. regalis* counted as dead did not decompose in galls after 6 weeks and

may have been alive but paralyzed by *B. celer* stings. No parasitoid cadavers were evident on dead or immobilized fly larvae. Although host killing may have caused us to underestimate host suitability in *R. fausta*, 67% of fly larvae survived and provided at least some opportunity for parasitoid reproduction. Documentation of host killing without reproduction under natural conditions should be considered part of the effective kill rate when natural-enemy efficacy is evaluated (Hoelmer and Kirk 2005).

Bracon celer's behavioral responses to *B. oleae* and non-target hosts showed a clear preference for searching and probing for *B. oleae* in olives. Searching and probing in olives were significantly higher than in non-target host material both in no-choice and choice comparisons (Figure 2). However, two factors, prior parasitoid experience on *B. oleae* in the colony and higher *B. oleae* density in the tests, may have led to underestimates of the behavioral responses toward the non-target species. The wasps we used were previously experienced with *B. oleae*. In their recommendations for parasitoid host-range testing in a no-choice assay, Withers and Browne (2005) note that evidence exists for a variety of outcomes resulting from prior parasitoid experience, and that levels of response toward unfamiliar hosts, compared with the familiar host in the familiar substrate, can be either enhanced, unchanged, or reduced due to prior experience. Nevertheless, parasitoid response is generally expected to be biased toward the familiar host and substrate combination, especially after the parasitoid successfully oviposits in it (Turlings, Wäckers, Vet, Lewis, and Tumlinson 1993). The second factor that may have led us to overestimate responsiveness by *B. celer* toward *B. oleae* was higher density of *B. oleae* than non-target hosts per cage. Numbers of *B. oleae* per cage averaged 1.7, 3.4, and 10.8 times higher than *C. succinea*, *P. regalis*, and *R. fausta*, respectively. This resulted from our attempts to present similar masses of target and non-target host material to wasps, while we underestimated infestation levels in non-target material. Higher host density may intensify volatile or contact chemical cues used by parasitoids during host location or cause increased parasitoid arrestment in the vicinity of hosts (Godfray 1994). If *B. celer* responds to concentrations of such chemical cues, and if it responds to cues from novel hosts, searching and probing on non-target species may have been underestimated in our study. Additionally, higher proportions of infested plant substrates or higher densities of larvae per plant structure may have elicited more searching and probing on olives by *B. celer*, which is likely to detect and respond to substrate vibrations caused by moving hosts, a host-location strategy common among parasitoids of mobile host stages hidden in plant substrates.

Although the level of response to non-target hosts may have been underestimated in our study, we were able to clearly demonstrate broad physiological host-range potential for *B. celer* due its ability to reproduce on the tephritine *P. regalis*. This result confirms expectations of low host specificity in ectoparasitic idiobionts (Shaw 1994; Althoff 2003). The low proportion of parasitized *P. regalis* and low proportion of female offspring relative to those produced on *B. oleae* may, however, indicate a reduced rate of acceptability and/or suitability of this non-target host, but prior parasitoid experience with *B. oleae* cannot be ruled out as a partial cause of reduced parasitism rates in the non-target host. In addition, the size of Cape ivy galls was variable and some larger galls may have prevented access by the *B. celer* ovipositor to all hosts inside. Lack of *B. celer* reproduction in *R. fausta*, despite ovipositor probes into cherries, indicates that this host is either not acceptable for oviposition or that it

is physiologically unsuitable for *B. celer* development. We cannot draw any conclusions about acceptability or physiological suitability of *C. succinea* (Tephritinae) to *B. celer*, because the wasps appeared to be unable to penetrate starthistle flower heads with their ovipositors. During trials females were observed repeatedly attempting to insert their ovipositors into the involucre surrounding the base of the florets, but they were unable to penetrate even the outermost layer of overlapping bracts. This in itself explains why no *B. celer* offspring were produced from this fly (Table 1).

Probing and attempted probing by *B. celer* into stem galls and flower heads clearly demonstrated that the parasitoid has the propensity to forage in diverse tephritid larval feeding substrates. This, combined with its broad host-range potential, suggests that *B. celer* poses a high risk of impacting non-target species. The threat to the beneficial species, *P. regalis*, is of special concern not only because *B. celer* can successfully parasitize it, but also because Cape ivy and olives grow in close proximity in coastal California, enhancing the possibility that parasitoids may enter Cape ivy patches either accidentally or in response to host cues. However, the importance of *B. celer*'s threat to *P. regalis* depends on whether the fly is released, establishes, and effectively controls Cape ivy. The threat to *C. succinea* and many of California's native tephritid fauna is tempered by the physical attributes of tephritid stem galls and flower heads, which hamper access by *B. celer* to hosts inside. Overlapping involucre bracts are characteristic of many asteraceous inflorescences, and might prevent access by the *B. celer* ovipositor to most hosts residing inside flower heads. Access through the soft florets at the top is limited in many inflorescences by the length of the *B. celer* ovipositor, which averages <3 mm long (Wang et al. 2008). Although *P. regalis* stem galls in Cape ivy are succulent and easily penetrated, most native tephritid stem galls are either tough and small or large and soft (D. Headrick, personal communication), presumably as adaptations by gallicolous flies to hinder access by native parasitoids, and which would also serve to restrict access by *B. celer*. Parasitoid ovipositor length is an important determinant of enemy-free space for tephritid larvae feeding deep within a plant structure, as demonstrated in the tephritid hosts *B. oleae* (Wang et al. 2008) and *Rhagoletis pomonella* Walsh (Feder 1995) in large fruit. Another biological factor limiting potential impact of *B. celer* or other imported tropical parasitoids on non-target species is the univoltinism of all native frugivorous tephritids and most gallicolous tephritids in California. These reflect the ephemeral nature of the plant resources available to them in the temperate climate of California, which likewise limits the window of opportunity for exploitation of third instar hosts. If *B. celer* succeeds in locating and reproducing on native fruit- or gall-formers, it is unlikely to establish populations on them, because the third instar is available for only a short period each year. *Bracon celer* is unlikely to diapause successfully, given its idiobiont lifestyle and tropical origin (contrast with some tropical koinobiont tephritid parasitoids that can diapause [Aluja, López, and Sivinski 1998]).

Compared with other braconids evaluated at the University of California, Berkeley, Quarantine, *B. celer* poses a higher risk to non-target species than *P. lounsburyi*, which did not probe or reproduce in non-target hosts in tests identical to those performed on *B. celer* (Daane et al. 2008). *Psytalia lounsburyi* has been approved for field release, in part due to its low risk of harming non-target species (Daane et al. 2008). The generalist egg-pupal parasitoid *Fopius arisanus* (Sonan), a

braconid that favors frugivorous tephritids, reproduced in *B. oleae* but failed to probe or reproduce in *C. succinea* or *P. regalis* (Sime, Daane, Wang, Johnson, and Messing 2008), indicating a narrower physiological and/or ecological specificity than *B. celer*. Its capacity to attack California's native frugivores has not been tested but its likelihood of encountering and establishing on them is limited by the same temporal and geographic barriers that hinder *B. celer* in California (Sime et al. 2008). *Fopius arisanus*, however, is less limited by physical barriers presented by host plants because of its long ovipositor and preference for the egg stage, which is laid by tephritids just below the plant surface (Sime et al. 2008).

We confirmed that *B. celer* has a broad physiological host range, and determined that it is capable of searching for hosts in diverse plant substrates. The tests were made under severe confinement and are therefore very conservative. It is likely that *B. celer* will be shown to have a narrower host range in the field. For example, we could find no reports of *B. celer* attacking *P. regalis* in South Africa, although the two species overlap in geographic distribution. Nevertheless, we prioritized release efforts to focus on other *B. oleae* parasitoids, such as the koinobiont *P. lounsburyi*, which were assessed to have less non-target risk in California (Daane et al. 2008). Future consideration of the importation and release of *B. celer* will be made after determining the effect of more specific parasitoids and the need for *B. oleae* biological control in California.

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